NICEATM and ICCVAM Genetic Toxicity Working Group comments on the draft Test Guideline (TG) 487: *In vitro* mammalian cell micronucleus test (MNvit) and on the draft *Performance Assessment EHS Monograph*

The cytotoxicity study data provided by the EU and US laboratories, generated November 2008 to July 2009, provide adequate evidence that the measures of cytotoxicity used in the third draft of TG 487, i.e., selection of a top concentration based on the two alternative measures, relative population doubling (RPD) or relative increase in cell counts (RICC). Either of these alternatives is sufficient to detect a genotoxic compound, compared to use of the more traditional survival measure, relative cell count (RCC). The choice of cytotoxicity metric did not change the calls on the 14 compounds evaluated in the trial. There were scattered negative calls in some labs with some agents, but in general, all of these compounds were scored correctly as positives. Therefore, the proposed TG 487 is acceptable as written, and it is recommended for final approval as per the conditions stated at the April 2008 WNT meeting.

The study did, however, confirm the suspicion of US experts that, relative to using RCC to set the top dose, the use of RPD or RICC would generally lower the top dose used for further testing, and this could result in a less conservative test. The counter argument is that such lower doses may better protect human health, which is a point that has not been adequately established. However, without any data to substantiate an effect on safety assessment, setting the top dose as that giving 55 +/- 5% cytotoxicity by RPD or RICC is considered acceptable.

RPD is preferred to RICC based on the study data and the fact that for radiation kinetics, cell inactivation/cytotoxicity is better described as a log function of dose than as a linear one. For chemical agents that have inactivation kinetics similar to radiation (which include at least simple alkyating agents and PAH and aryl amine derivatives), the use of a linear function results in an overestimate of cytotoxicity for the assay, especially at doses approaching the 55 +/-5% limit. In addition to identifying almost all of the positive control compounds, including those added to challenge the sensitivity of the system, RPD has the advantage of properly modeling cell growth, which is recognized as an exponential function.

Previous attempts to deal with the cytotoxicity issue lacked the supporting data for this change. For example, IWGT in 2005 suggested that a positive call required an increase not only at the 50% cytotoxicity level but also at more moderate levels. That IWGT suggestion is NOT captured by this test guideline; however, the insensitivity of RICC or RPD is balanced by slightly raising the level of cytotoxicity from "at least 50%" to "55 +/- 5%" and by interpreting an increase at that dose as a positive assay, no matter what happens at lower levels of toxicity. Despite limitations in the validation data set for RICC and RPD, it is far better than the previous concept that the in vitro chromosome damage assays are "too sensitive".